Evaluation of a Novel Kit (TF-Test) for the Diagnosis of Intestinal Parasitic Infections

Jancarlo Ferreira Gomes,1,2 Sumie Hoshino-Shimizu,2,3* Luiz Cândido S. Dias,1 Ana Júlia S.A. Araujo,4 Vera L.P. Castilho,5 and Fátima A.M.A. Neves6

1Department of Clinical Parasitology, State University of Campinas, São Paulo, Brazil
2Orion Research Center for Biotechnology, São Paulo, Brazil
3University of São Paulo, São Paulo, Brazil
4Department of Parasitology, Clinical Hospital, University of São Paulo, São Paulo, Brazil
5Laboratory of Parasitology, Clinical Hospital, University of São Paulo, São Paulo, Brazil
6Laboratory of Clinical Analysis, Clinical Hospital, Paulista State University, Botucatu, São Paulo, Brazil

Intestinal parasitic infections are currently a source of concern for Public Health agencies in developing and developed countries. Since three ovum-and-parasite stool examinations have been demonstrated to provide sensitive results, we designed a practical and economical kit (TF-Test) that is now commercially available (Immunoassay Com. Ind. Ltda., São Paulo, Brazil). This kit allows the separate collection of three fecal specimens into a preservative solution. The specimens are then pooled, double-filtered, and concentrated by a single rapid centrifugation process. The TF-Test was evaluated in four different laboratories in a study using 1,102 outpatients and individuals living in an endemic area for enteroparasitosis. The overall sensitivity found using the TF-Test (86.2–97.8%) was significantly higher (P<0.01) than the sensitivity of conventional techniques such as the Coprotest (NL Comércio Exterior Ltda, São Paulo, Brazil) and the combination of Lutz/Hoffman, Faust, and Rugai techniques (De Carli, Diagnóstico Laboratorial das Parasitoses Humanas. Métodos e Técnicas, 1994), which ranged from 48.3% to 75.9%. When the above combined three specimen technique was repeated with three specimens collected on different days, its sensitivity became similar (P>0.01) to that of the TF-Test. The kappa index values of agreement for the TF-Test were consistent (P<0.01), being higher and ranking in a better position than conventional techniques. The high sensitivity, cost/benefit ratio, and practical aspects demonstrate that the TF-Test is suitable for individual diagnosis, epidemiological inquiries, or evaluation of chemotherapy in treated communities. J. Clin. Lab. Anal. 18:132–138, 2004. © 2004 Wiley-Liss, Inc.

Key words: TF-Test; parasite-enrichment process; pooled three fecal specimen examinations; intestinal parasitic infections

INTRODUCTION

The prevalence of intestinal parasitic infections is high, mostly in tropical and subtropical developing countries. The World Health Organization (1) estimates that about 3.5 billion people in the world have single or multiple intestinal parasitoses. Also, intestinal parasitoses have become a public health concern in developed countries because of the increase in intercontinental travel and immigration and the increase in the number of immunocompromised subjects. Physicians in developed and developing countries are now requesting frequent stool examinations for intestinal parasites, or they are recommending at least one stool examination per year, especially for immunocompromised patients (2).

The conventional techniques involving ovum-and-parasite (O&P) examination have been proven to miss

Grant sponsor: FAPESP; Grant number: 99/06228-4; Grant sponsor: Immunoassay Ind. Com. Ltda.

*Correspondence to: Sumie Hoshino Shimizu, Dept. Clinical and Toxicological Analyses, Faculty of Pharmaceutical Sciences, University of São Paulo. Av. Prof. Lineu Prestes, 580, Bloco 17, Cidade Universitária, CEP 05508-900, São Paulo, Brazil.
E-mail: sshimizu@usp.br

Received 15 August 2003; Accepted 26 September 2003
DOI 10.1002/jcla.20011
Published online in Wiley InterScience (www.interscience.wiley.com).
many pathogenic parasites (3). Thus, in order to confirm
the presence of intestinal parasites, it has been shown
that three stool specimens are needed in routine
laboratory examinations (4). In addition, in a high-
prevalence setting, at least two examinations have been
considered to be necessary (5,6).

In an attempt to improve the efficiency of stool
examination techniques, several concentration proce-
dures have been suggested, either by pooling three
formalin-preserved stool specimens for conventional
techniques (7) or by using some commercially available
concentration device suitable for concentrating one
stool specimen (8).

We have recently designed a practical and economical
kit (TF-Test) that is now manufactured by Immunoas-
say Com. Ind. Ltda. (São Paulo, Brazil). This kit allows
the collection of stool specimens separately into a
preservative solution on three different days. The
specimens are then pooled by a 1-min centrifugation
process, double-filtered, and concentrated before para-
site identification by standard light microscopy.

In this study, evaluation of the TF-Test’s diagnostic
features is presented in comparison with conventional
techniques for an O&P examination, performed at four
reference laboratories belonging to universities located
in different cities in the State of São Paulo, Brazil.

MATERIALS AND METHODS

Stool Specimens

A total of 1,102 subjects were studied and three stool
specimens were collected from each individual for the
TF-Test. In addition, one more specimen was collected
for the conventional technique used in each of three
laboratories in the State of São Paulo. In the first
laboratory (A) located in the City of Taubaté (Labora-
tory of Parasitology, Department of Biology, University
of Taubaté), specimens were collected from inhabitants
of the rural zone, an endemic area for enteroparasitosis.
In the second laboratory (B), in the City of Botucatu
(Laboratory of Clinical Analyses, Clinical Hospital,
Paulista State University), specimens were collected
from outpatients. In the third laboratory (C), in the
City of Campinas (Laboratory of Clinical Parasitology,
School of Medical Sciences, State University of Campi-
nas), specimens were also collected from outpatients.
In the fourth laboratory (D) located in the City of
São Paulo (Laboratory of Parasitology, Clinical
Hospital, Medical School, University of São Paulo),
three stool specimens were collected for the TF-Test and
three specimens for each of the three standard O&P
techniques.

This project was submitted to the Ethics Committee
of each university, and written informed consent was
obtained from the subjects who agreed to participate in
the project.

TF-Test

The parasite enrichment device (Fig. 1) was made of
disposable and recyclable plastic (polypropylene) and
consisted of three vials for specimen collection, each
containing 5 mL preservative solution that displayed a
fill-to line in order to permit the patient to visually check
whether or not the amount of collected stool specimen
was adequate. Several fixatives were available, but all
participating laboratories used the traditional formalin-
buffered solution. About 1.0 g stool specimen was
collected from each vial with a scoop connected to the
cap, and three specimens were obtained on alternate
days, or within a week. After collecting each specimen,
the patient was asked to homogenize the vial by
moderate shaking in order to ensure parasite fixation
and the maintenance of their morphological structure.
In the laboratory, 2 mL ethyl-acetate and a drop of
detergent were added to each vial. After homogeniza-
tion, all the vials were coupled to a double-filtration
system attached to a conical centrifuge tube and
submitted to a 1-min centrifugation (500 × g). The
centrifuge tube was then detached from the system,
the supernatant was discarded, 10 drops of saline were
added to the sediment, and one drop of the sediment
suspension was placed on a slide. However, depending
on sediment concentration, one additional drop of saline
was added before examination for the purpose of
detecting parasites by routine microscopy.

Conventional Techniques for Stool Examination

The conventional techniques for O&P examination
differed according to the laboratory: laboratories B and
C used only the Coprotest, previously known in Brazil
as Total test (NL Comércio Exterior Ltda.) (9), which
consists of one vial for collecting one stool specimen;
laboratory A used a combination of the Coprotest and
Kato-Katz (10); and laboratory D used a combination
of the Lutz/Hoffman, Faust, and Rugai techniques (11).

Statistical Analysis

The results of the TF-Test were evaluated in
comparison with those of the routinely used techniques.
The positivity found by the combination of all
techniques used in each laboratory was considered to
be the reference value. The positivity found by the
TF-Test was compared with the standard techniques
by the z-test of proportions (12). Also, 95% confidence
intervals were calculated for sensitivity or specificity
(13). The efficiency of the techniques was also
determined in terms of the kappa (κ) index of agreement (14) by testing the consistency of κ (15) and its rank, based on the strength of the κ index (16), and defined as follows: poor for values ranging from 0 to 2.0, slight for values from 0.21 to 0.40, moderate for values from 0.41 to 0.60, substantial for values from 0.61 to 0.80, and almost perfect for values from 0.8 to 1.0.

RESULTS

TF-Test and Conventional Techniques

The results regarding the number of infected subjects, type of infection (single or multiple), and the number of different parasite species detected by the TF-Test and by the conventional techniques are presented in Table 1. Each laboratory had its own reference data corresponding to the overall positive and negative results obtained by the combination of the conventionally used technique(s) including the TF-Test.

In laboratories A, B, C, and D, intestinal parasitic infections were found at frequencies ranging from 14.3–60.5%. The TF-Test detected a total of 406 (36.8%) subjects with single or multiple enteroparasitosis, a significantly higher number (obtained $z = 3.25$; critical $z = 2.57$; $P < 0.01$) than the 334 (30.3%) infected subjects detected by conventional techniques. Completely negative results were observed in 660 (59.8%) subjects by all the techniques used with 31.4% (207) of these being from laboratory A, 38.9% (256) from laboratory B, 14.2% (94) from laboratory C, and 15.5% (102) from laboratory D.

Parasite Species

In 443 subjects with single, double, triple, or multiple (≥4) infections, a total of 807 intestinal parasitic infections were detected. The positivity found by the TF-Test was 88.1% (711), which was significantly higher (obtained $z = 11.5$; $P < 0.01$) than the positivity of 63.7%
(514) found by conventional techniques (Table 2). In laboratory A, the data provided by the Kato-Katz (10) technique were not included because the positive results were very few and agreed with the Coprotest. In Table 2, the overall protozoan and helminth species identified by the TF-Test and conventional techniques are also presented.

Protozoan infections were observed in 56.7% (625/1102) of subjects, with their frequency being considerably higher than that of helminth infections (16.5%, 182/1102) in four laboratories. Statistical analysis confirmed that the frequency of protozoan infections was significantly higher than that of helminth infections (obtained $z = 12.0; P < 0.01$).

### Sensitivity and Specificity of the Techniques

The sensitivity of the TF-Test was calculated on the basis of the ability of a technique to detect infections caused by different parasite species in comparison with the reference data. Table 3 shows that the TF-Test presents significantly higher sensitivity (obtained $z = 3.8; P < 0.01$) than the conventionally used techniques, but in laboratory B, the TF-Test and Coprotest presented similar sensitivity (obtained $z = 1.25; P > 0.01$) due to the low number of positive results. A total of 300 outpatients were studied in laboratory B and only 43 of them were infected with 46 parasite species. Also, in terms of the confidence intervals (95%), the sensitivity of the TF-Test (69.0–91.0%) overlapped the sensitivity of the Coprotest (57.0–83.1%), indicating no difference between these two techniques.

### Table 1. Results obtained in the study of 1,102 subjects by the TF-Test and by conventional techniques in four different laboratories in the State of São Paulo, Brazil

<table>
<thead>
<tr>
<th>Laboratory (no. of studied subjects)</th>
<th>Technique</th>
<th>No. of subjects with single or multiple infections (%)</th>
<th>Single no.</th>
<th>Double no.</th>
<th>Triple no.</th>
<th>Multiple no.</th>
<th>Total no. of parasite Infections detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (309)</td>
<td>TF-Test</td>
<td>100 (32.4)</td>
<td>76</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>Coprotest</td>
<td>80 (25.9)</td>
<td>62</td>
<td>14</td>
<td>3</td>
<td>1</td>
<td>104</td>
</tr>
<tr>
<td>B (300)</td>
<td>TF-Test</td>
<td>35 (11.7)</td>
<td>32</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Coprotest</td>
<td>31 (10.3)</td>
<td>29</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>C (238)</td>
<td>TF-Test</td>
<td>137 (57.6)</td>
<td>79</td>
<td>26</td>
<td>21</td>
<td>11</td>
<td>242</td>
</tr>
<tr>
<td></td>
<td>Coprotest</td>
<td>112 (47.1)</td>
<td>59</td>
<td>25</td>
<td>14</td>
<td>14</td>
<td>208</td>
</tr>
<tr>
<td>D (256)</td>
<td>TF-Test</td>
<td>134 (52.3)</td>
<td>42</td>
<td>42</td>
<td>31</td>
<td>18</td>
<td>297</td>
</tr>
<tr>
<td></td>
<td>Three tec.</td>
<td>111 (43.4)</td>
<td>70</td>
<td>32</td>
<td>3</td>
<td>6</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>Positives</td>
<td>154 (60.2)</td>
<td>49</td>
<td>50</td>
<td>30</td>
<td>25</td>
<td>349</td>
</tr>
</tbody>
</table>

*Multiple means ≥4 parasitic infections.

In affected subjects.

Combination of the Lutz/Hoffman, Faust, Rugai techniques.

### Table 2. Parasite species identified by the TF-Test and conventional techniques in the study of 1,102 subjects in four different laboratories in the State of São Paulo, Brazil

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>TF-test (positive no.)</th>
<th>Routine technique (positive no.)</th>
<th>Total positivity (no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protozoan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. nana</td>
<td>13.5% (149)</td>
<td>9.6% (106)</td>
<td>15.7% (173)</td>
</tr>
<tr>
<td>E. coli</td>
<td>14.0% (154)</td>
<td>9.5% (104)</td>
<td>15.1% (166)</td>
</tr>
<tr>
<td>B. hominis</td>
<td>10.1% (111)</td>
<td>6.8% (75)</td>
<td>11.7% (129)</td>
</tr>
<tr>
<td>G. lamblia</td>
<td>8.2% (90)</td>
<td>5.5% (61)</td>
<td>8.4% (93)</td>
</tr>
<tr>
<td>I. butschlii</td>
<td>2.5% (28)</td>
<td>1.5% (17)</td>
<td>3.0% (33)</td>
</tr>
<tr>
<td>E. histolytica/dispar</td>
<td>1.6% (18)</td>
<td>0.8% (9)</td>
<td>1.9% (21)</td>
</tr>
<tr>
<td>E. hartmanni</td>
<td>0.3% (3)</td>
<td>0.5% (5)</td>
<td>0.7% (8)</td>
</tr>
<tr>
<td>C. mesnili</td>
<td>0.1% (1)</td>
<td>0.2% (2)</td>
<td>0.2% (2)</td>
</tr>
<tr>
<td>Helminth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ancylostomatidae</td>
<td>5.3% (58)</td>
<td>4.7% (52)</td>
<td>5.7% (63)</td>
</tr>
<tr>
<td>T. trichiura</td>
<td>4.1% (45)</td>
<td>3.2% (35)</td>
<td>4.4% (48)</td>
</tr>
<tr>
<td>S. stercoralis</td>
<td>2.8% (31)</td>
<td>2.0% (23)</td>
<td>3.6% (40)</td>
</tr>
<tr>
<td>S. mansoni</td>
<td>1.1% (12)</td>
<td>1.1% (13)</td>
<td>1.6% (18)</td>
</tr>
<tr>
<td>E. vermiculares</td>
<td>0.8% (9)</td>
<td>0.9% (10)</td>
<td>1.0% (11)</td>
</tr>
<tr>
<td>H. nana</td>
<td>0.2% (2)</td>
<td>0.2% (2)</td>
<td>0.2% (2)</td>
</tr>
<tr>
<td>Total</td>
<td>64.5% (711)</td>
<td>46.6% (514)</td>
<td>73.3% (807)</td>
</tr>
</tbody>
</table>

*Coprotest or a combination of the Lutz/Hoffman, Faust, and Rugai techniques.

Maximum specificity (100%) was found for all the techniques in all four laboratories. In laboratory D, the sensitivity of the Lutz/Hoffman, Faust, and Rugai techniques, each alone or in combination (Table 4), was found to increase significantly (obtained $z = 2.7; P < 0.01$) after repeating two and three stool collections and O&P examinations. Also, the sensitivity of the
combination of these techniques improved gradually and significantly achieving 89.4%, which did not differ significantly (obtained $z = 1.58; P < 0.01$) from the TF-Test (85.1%) in the same laboratory.

**Kappa ($\kappa$) Index of Agreement**

The $\kappa$ index indicates the agreement of positive and negative results between a technique under evaluation and the reference data, considered here as true diagnoses. In all laboratories, the TF-Test ranked in a better position than the conventional techniques (Table 5). All the $\kappa$ indices obtained were consistent because the obtained $z$-values were all higher than 3.89 ($P < 0.01$).

**DISCUSSION**

The techniques currently used for O&P examination are usually highly specific, but tend to yield false-negative results. Thus, the improvement of these techniques for the identification of parasites in stool specimens is imperative in order to obtain sensitive results. Most Public Health laboratories from developing countries are interested in stool examination techniques because they focus on the unequivocal diagnosis of intestinal parasitosis at low cost. Thus, in an attempt to satisfy the expected diagnostic features, the TF-Test was designed to deal with this matter.

Parasitologic techniques provide true diagnoses since the causative agent is demonstrated directly, differing from other more sophisticated techniques such as immunoassays. Though the high sensitivities of immunoassays are recognized, the positive and negative results obtained with them are interpreted in terms of probability. Also, it has been reported (17) that, in some instances, multiple stool specimen analyses were required to improve the diagnosis of enteroparasitosis using these assays.

There are different ways to evaluate the diagnostic performance of a technique. In the present interlaboratory evaluations we focused on some procedures with which we have become familiar considering the development and evaluation of new reagents (18), quality control analysis (19), and comparison of techniques (20) for the diagnosis of some parasitic and viral infections.
In general, the diagnostic performance of the TF-Test was better than that of conventional techniques. This finding was expected, since pooling three specimens from different days (7), or even three specimens from the same day (21), is a process that concentrates or enriches O&P. The data in Table 4 illustrate the increase in parasite yields, even with less sensitive conventional techniques, using two and three repeats. In the combination of these techniques with three repeats, the final sensitivity became as high as that of the TF-Test since 144 (41.3%) subjects with previously undetected parasites became positive. Depending on the frequency of parasitic infections and the technique used, it has been reported that three O&P examinations yield 22.7% (3) to 41.7% (4, 5) additional positive results.

The evaluation of the TF-Test was performed in laboratories showing different degrees of positivity: 1) low frequency of intestinal parasitic infections (B); 2) high frequency of infections (C and D); and 3) intermediate frequency of infection (A), in an endemic zone for enteroparasitosis. In these laboratories, there were single, double, or multiple parasitic infections, with the prevalence of a higher frequency of protozoan infections than helminth infections. This profile, however, is consistent with the epidemiological data obtained over the last 10 years at different localities in the State of São Paulo (22, 23).

In the second type of evaluation, we analyzed the efficiency of the techniques based on the χ index of agreement for positive and negative results in relation to the reference data. The χ index better defines the diagnostic performance of different techniques rather than providing a simple estimate in terms of the percent agreement or disagreement.

In laboratory B, although the sensitivity of the TF-Test did not significantly differ from that of the Coprotest, the χ index of the TF-Test was found to be significantly higher than that of the Coprotest. This can be explained by the fact that the χ index dealt with a large number of negative results (257) in addition to the positive results used for the evaluation of sensitivity.

The χ index of this technique was ranked as almost perfect for laboratories A and B and substantial for laboratories C and D. Possibly in the latter laboratories there were some factors influencing the efficiency of the new technique such as: 1) different concepts and procedures introduced for both patients and laboratory personnel; 2) the need of more technical skill similar to that acquired for the routinely used technique(s); and 3) variance among technicians, since more than two technicians participated in those laboratories where the frequency of multiple parasitic infections was high. However, the present findings speak in favor of the new technique.

The TF-Test proved to be flexible, providing several options for the collection of stool specimens such as: 1) the use of a desired preservative solution, or the collection of one specimen without and the other two with a preservative solution in cases in which bacterial culture is requested; 2) when staining procedures are required in the search for some coccidian oocysts in immunocompromised subjects; or 3) even to collect three specimens from the different parts of the same stool (21); etc. Also, in treated patients or in programs for enteroparasitosis control, this technique may be useful because of its high sensitivity, considering that antibody detection is ineffective for treated patients.

The TF-Test was designed to improve the parasitologic examination of stool specimens and, in this respect, the data obtained demonstrate that the objective was attained. Also, the information collected through questionnaires filled out by the users, i.e., outpatients and laboratory staff (data not shown) confirmed its useful features. The main advantages of the TF-Test are high sensitivity, suitable cost/benefit ratio, practical and easy specimen handling and processing in the laboratory, a small laboratory area required for working with it, and rapidly obtained results.

Thus, the high sensitivity and economical and practical aspects of the TF-test show that the test is applicable to individual diagnosis and epidemiological surveys. Moreover, this technique may contribute efficiently to the monitoring of chemotherapy during the follow-up of populations treated in programs of enteroparasitosis control.

REFERENCES